PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PAM-004-PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/09980	International filing date *(day/month/year)* 01.09.2003	Priority date *(day/month/year)* 02.09.2002
International Patent Classification (IPC) or both national classification and IPC C12Q1/68		
Applicant PAMGENE B.V. ET AL.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
26.03.2004	14.10.2004
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Botz, J Telephone No. +31 70 340-4513

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/09980

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-24 as originally filed

Claims, Numbers

1-20 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4,5,7,9-11
	No:	Claims	1-3,6,8,12-20
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-20
Industrial applicability (IA)	Yes:	Claims	1-20
	No:	Claims	

2. Citations and explanations

see separate sheet

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. **Reference is made to the following documents:**

D3: WO 01 34842 A (STRIZHKOV BORIS N ;MIKHAILOVICH VLADIMIR (US); MIRZABEKOV ANDREI () 17 May 2001 (2001-05-17)

D5: WO 99 02266 A (AKZO NOBEL NV ;DAMME HENDRIK SIBOLT VAN (NL); KREUWEL HERMANUS JOH) 21 January 1999 (1999-01-21) cited in the application

D7: VAN BEUNINGEN ET AL: "Fast and specific hybridization using low-through microarrays on porous metal oxide" CLINICAL CHEMISTRY, AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY. WINSTON, US, vol. 47, no. 10, 12 December 2001 (2001-12-12), pages 1931-1933, XP002200111 ISSN: 0009-9147

2. **NOVELTY (Article 33 (2) EPC)**

2.1 The present application does not meet the criteria of **Article 33(1) PCT**, because the subject-matter of **claims 1 - 3, 6, 8, 12 - 20** is not new in the sense of **Article 33(2) PCT**.

2.2 The porous substrate containing the microchannels in the underlying application is described on page 4 as referring either to a pore being an opening or a microchannel, by which matter may either be absorbed *or* passed through. The porous substrate in **D3** is described as resembling micro-miniaturized test tubes and is considered to meet the description of the underlying application. Prior art **D3** provides a technique which is not described to be limited to the identification of on-chip-amplification of pre-determined analyte molecules, neither is the underlying application. On page 8 of **D3**, the problem of detection limits of low-concentration analyte samples by integrating an amplification step in the microarray analysis of the analyte is addressed, when it is stated in the last paragraph of said page, that as little as 100 DNA molecules are required to perform such an analysis. The term "target molecule" is formulated in the underlying application relatively broadly (page 13), when refering to a molecule capable of binding to an analyte molecule. The term therefore comprises the primer-function of cited prior art **D3**. The primers in **D3**

are fluorescently labelled and the amplified product is monitored in real time with a fluorescent microscope. The specificity of the reaction was tested by hybridization of the extended immobilized primers with the labeled reverse primer or internal probe, c.f. page 5. The underlying application explicitly mentions fluorescent reporter system to be used in the method of the invention, c.f. page 14.

2.3 **D3** is considered novelty destroying to **claims 1 - 3, 6, 8, 12 - 20,** care also for page 7, "Detailed description of the invention" to page 14, in particular for page 9, line 22 to page 10 line 19, page 11, line 28 to page 12 line 21, Examples 1, 2 and 6, Figure 4, page 23, line 3 to line 22, whole section on "Materials and Methods". The acrylamide-matrix / the gel-pads are considered as a permeable substrate and are therefore novelty-destroying to claim 9.

3. INVENTIVE STEP (Article 33 (3) PCT)

3.1 Document **D3** is considered to represent the **most relevant state of the art** for claims 1 - 20 and discloses PCR amplification on microarrays of gel immobilized oligonucleotides, c.f. discussion on novelty further above.

3.2 The subject-matter of claims 1 - 20 **differs** in that the method of analyte nucleic acid identification of the underlying application is performed on a porous substrate, namely a flow-through microarray, composed of ***aluminum oxide***.

3.3 The **effect** of the use of said flow-through microarray would be a reduction in incubation time, due to a minimization of diffusion. It allows high-throughput microarray analysis and furthermore integrated amplification-hybridization-detection of sample analytes.

3.4 The **problem to be solved** by the present invention would therefore be regarded as providing a more advanced microarray structure for performing nucleic acid analysis-assays.

3.5 This **solution could not however be considered as involving an inventive step** for the following reasons:

3.5.1 The flow-through microarray bearing a porous substrate, said porous substrate consisting of aluminum-oxide and being composed of microchannels, already exists in the

prior art: **D7** introduces such a structure, c.f. the whole document. This porous microarray is suited for all kinds of nucleic acid analysis assays / clinical diagnostics and in particular for nucleic acid hybridization and real time detection, c.f. last paragraph on page 1933. **D5**, originating from the same author as **D7**, also describes and details even further said porous microarray composed of microchannel containing aluminum-oxide.

3.5.2 Since both **D3** and **D7** (or: **D5**) are dealing with nucleic acid analysis assays on solid supports and are therefore located in the same technical field, it would have been obvious for the skilled in the art to combine the teachings of both documents and to arrive at the solution provided by the applicant without the exercise of inventive skill.

3.5.3 The use of fluorescent quenching systems such as the application of molecular beacons and real-time determination by means of e.g. Taqman or Light Cycler are state of the art. Isothermal amplification systems such as NASBA or TMA are also known to the skilled person, who would therefore regard it as a normal options to comprise these features within the method of the underlying application, c.f. claims 4 and 5.

3.5.4 In view of the above, the present application does not meet the requirements of **Article 33 (1) PCT**, because the subject-matter of **claims 1 - 20** does not involve an inventive step in the sense of **Article 33 (3) PCT**.